A Test of the Cognitive Self-Medication Hypothesis of Tobacco Smoking in Schizophrenia

Supplemental Information

Supplemental Methods

Additional Detail on Study Participants

All healthy control subjects (HCS) were cigarette smokers. Two participants with schizophrenia (SCZ) smoked cigarillos, the rest cigarettes.

Patient diagnosis was established from a Structured Clinical Interview for DSM-IV (SCID) combined with a review of patient medical records. Sixteen of the SCZ were clinically stable outpatients, and one was an inpatient at the Treatment Research Unit of the Maryland Psychiatric Research Center. Medication status or dosage had not changed in the preceding four weeks. The one unmedicated SCZ had been unmedicated for the preceding three years. HCS were recruited from the community via random digit dialing and word of mouth or had been past participants of studies at the National Institute on Drug Abuse – Intramural Research Program (NIDA-IRP). HCS had no Axis 1 or 2 diagnoses as established by a SCID, had no self-reported family history of psychosis, and were not taking any psychotropic medication.

Before participants signed the consent form, the investigator reviewed its content with the volunteer and answered any questions. For SCZ, the investigator, in the presence of a third-party witness, formally evaluated basic understanding of study demands, risks, and what to do if experiencing distress or to end participation, prior to allowing the volunteer to sign the consent form. Because this was a collaborative study with NIDA-IRP, the NIDA-IRP IRB also reviewed and approved the study protocol.

Additional Detail on Study Procedures

Nicotine and placebo patches were applied to the upper back. The placebo patch was a size-matched band aid. Each patch was covered by a generous amount of tape to help equate potential somatosensory differences related to their adherence. The patches were applied by a study nurse who was not involved in any other testing procedures to ensure the experiment was conducted in a double-blind manner.

The 3.5-hour absorption period was chosen to allow sufficient time for stable patch-derived blood nicotine concentrations to be reached (1-3). At the same time, 3.5 hours of smoking abstinence could be expected to result in a substantial reduction in smoking-derived blood nicotine levels (4;5), while evoking at most minimal withdrawal-related cognitive deficits (6;7).

Nicotine withdrawal was measured by (a) the 8-item Minnesota Nicotine Withdrawal Scale (MNWS (8)), on which participants rated the items “angry, irritable, frustrated”, “anxious, nervous”, “depressed mood, sad”, “desire or craving to smoke”, “difficulty concentrating”, “increased appetite, hungry”, “restless”, and “impatient” on a scale from 0 (= none) to 4 (= severe), and (b) a list of bidirectional 9-point scales shown to be sensitive to mood changes induced by tobacco deprivation (9): tense/relaxed, nervous/calm, energetic/tired, alert/drowsy, contented/irritated, satisfied/dissatisfied. These bidirectional scales were coded such that larger values represent more negative ratings. Values were averaged across scales (results are presented below).

Analysis of Nicotine and Its Metabolites in Plasma

Nicotine, cotinine, trans-3'-hydroxycotinine (OH-cotinine) and norcotinine were measured concurrently in 0.5 mL plasma specimens by liquid chromatography tandem mass spectrometry (LCMSMS). Two mL 0.1% formic acid were added to plasma specimens and centrifuged at 4,000x g for 5 min at 4°C. Supernatants were submitted to solid phase extraction with Strata-XC cartridges (Phenomenex, San Jose, CA). Conditioning was performed with methanol and water, and washing with 0.1 M acetic acid and methanol. The final elution was accomplished with 3% NH_4OH in methanol. Samples were reconstituted in 100 µL of mobile phase and 20 injected into the LCMSMS.

LCMSMS analysis was performed with a Shimadzu liquid chromatography system (Shimadzu Corporation, Columbia, MD) interfaced to a 3200 QTrap (AB Sciex, Foster City, CA) with a Turbo V ESI source. The Shimadzu system consisted of a LC-20AD binary pump, DGU-20A3 degasser, SIL-20AD autosampler and CTO-10AC column oven. The chromatographic separation was achieved with a Synergi Polar-RP 100A, 100 x 2 mm, 4 µm, with a 4 x 2 mm identically packed guard column (Phenomenex, Torrance, CA). Gradient elution was with mobile phase A (1 mM ammonium formate pH 3.3 with 0.1% formic acid) and mobile phase B (acetonitrile) at a flow rate of 0.3 mL/min. The initial mixture (94A:6B) was maintained for 3 min, then mobile phase B was increased to 60% at 5 min and held for 3 min. The mixture returned to the initial conditions at 10 min, followed by 2 min equilibration. The total run time was 12 min. Mass spectrometric data were acquired in positive electrospray ionization mode with the following source parameters: IonSpray voltage 3,000 V; temperature 450°C; curtain gas 35; ion source gas1 50 and ion source gas2 50. Data were recorded in multiple reaction monitoring mode. The following transitions were monitored (quantification transition in bold):

163.2 > 132.2 and 163.2 > 84.2 for nicotine; **177.2 > 80.1** and 177.2 > 98.1 for cotinine; **193.2 > 80.2** and 193.2 > 134 for OH-Cotinine; **163.2 > 80.2** and 163.2 > 118.2 for norcotinine; **167.2 > 136.1** and 167.2 > 121 for Nicotine-d_4; **180.2 > 80.2** and 180.2 > 101.2 for Cotinine-d_3; **196.2 > 79.9** and 196.2 > 134.1 for OH-Cotinine-d_3; and **167.2 > 84.2** and 167.2 > 139.2 for norcotinine-d_4. Linearity ranges with 1/x weighting for nicotine and $1/x^2$ for metabolites were from 1 to 500 ng/mL for cotinine, OH-cotinine and norcotinine, and from 2.5 to 500 ng/mL for nicotine. The lower limit of quantification was 1 ng/mL for cotinine, OH-cotinine and norcotinine, and 2.5 ng/mL for nicotine, and the limit of detection was 0.5 ng/mL for cotinine, OH-cotinine and norcotinine, and 1 ng/mL for nicotine. Assay accuracy at low, medium and high QCs was 90.1-103.5% (n = 20) and imprecision was 4-13.8% (n = 20). All analytes were stable after 48 h in the autosampler.

Additional Detail on the Cognitive Tasks

The attention tasks were completed in a dimly illuminated room on a 17” CRT monitor with a 60 Hz refresh rate. Eye-tracking was performed during the Spatial Attentional Resource Allocation Task (SARAT) to monitor central fixation, using an EyeLink 1000 eye-tracking system operating at 2000 Hz (SR Research Ltd., Mississauga, Ontario). The eye-tracker consisted of an infrared light source and video camera, providing an image of the participant’s right eye. Participants rested their heads on a chin and forehead rest at 70 cm viewing distance from the monitor.

SARAT

The peripheral target locations were marked by placeholders. The central circle and placeholders formed a background that remained on display throughout the task, including inter-

trial intervals. With eyes directed at the center of the fixation cross, the center of the peripheral placeholders was positioned at an eccentricity of 12.5º. A trial began once continuous central fixation was maintained for 500 ms.

Upon detecting a target, participants pressed a button with their dominant index finger as quickly as possible. A target consisted of one of the placeholders filling with a checkerboard of grey and white squares of 3 x 3 pixels each. The contrast of the gray checks was 80% for the high-contrast targets and 20% for the low-contrast targets. The cue remained on display until 500 ms after target offset. When two quadrants were cued, they were always adjoining. Cue-only trials were identical to the other trials, except that no target was presented during the 500-ms target interval. All trials were followed by a 1500-ms intertrial interval. There were 336 valid trials (56 x 1/2/4 cued locations x high/low target contrast), 56 invalid trials (14 x 1/2 cued locations x high/low contrast) and 42 cue-only trials (14 x 1/2/4 cued locations).

Singleton Distractor Task

The luminance of the green background was 7.215 cd/m^2. The size of the circle stimuli was 1.44° x 1.44°; the size of the diamonds was 1.51° x 1.51°. The size of the line segment inside the stimuli was 0.53° x 0.04°, and the line color was the same as the background. All non-target stimuli contained diagonal lines; the orientations were 22.5°, 67.5°, 112.5°, or 157.5°.

The red of the stimuli was isoluminant to the background, determined using a flicker fusion procedure. Four squares subtending 1.89° x 1.89° were presented against a gray background, 4.91° from a central fixation cross. The squares flickered between the green color used for the background during the search task and red at a rate of 60 Hz. The red value started at its minimum and was increased. Subjects were instructed to fixate centrally and report when the

apparent flicker between the red and green had been minimized. The isoluminant red value found using this procedure was used during the search task.

Supplemental Results

Additional Detail on Dependence Severity Indices

The trend toward lower Fagerstrom Test for Nicotine Dependence scores in SCZ was driven by fewer SCZ endorsing that they would most hate to give up the first cigarette in the morning, that they smoke more frequently during the first hours after waking, and that they smoke if they are so ill that they are in bed most of the day. The items regarding which cigarette they would most hate to give up, and smoking when ill, in particular, were found to have low factor loadings in SCZ (10).

When asked whether participants had experienced certain withdrawal symptoms when refraining from smoking, there were no group differences for craving, irritability, trouble concentrating, restlessness, impatience, disrupted sleep, increased eating, or feeling drowsy (p > 0.4 for all variables except drowsy, where p > 0.2). However, more SCZ endorsed experiencing anxiety [12 SCZ vs. 6 HCS; $c^2 = 4.25$, $p < 0.04$].

The State-Trait Anxiety Inventory

SCZ and HCS did not differ in trait anxiety [$t_{(32)} = 0.81$, $p > 0.4$]. State anxiety was higher in the placebo than the transdermal nicotine and ad libitum smoking condition [Figure S1; main effect of Drug in 2-factor analysis of variance (ANOVA): $F_{(2,62)} = 3.36$, $p < 0.05$]. State-anxiety tended to be higher in SCZ, [main effect of Group $p = 0.13$]. There was no interaction of Group with Drug [$p > 0.4$]. Thus, although more SCZ reported that they experienced withdrawal-induced anxiety (see above), SCZ and HCS averaged the same acute increase with nicotine deprivation.

Affect-Related Withdrawal Scales (9)

Figure S2 shows that negative affect increased over time in the placebo but not the nicotine patch condition. When analyzing the patch conditions by 3-factor ANOVA, an interaction of Drug with Time [$F_{(2,64)} = 3.23$, $p < 0.05$] confirmed this. The graph suggests that self-ratings of SCZ were again less sensitive to drug condition: the increase in the placebo condition was less pronounced than in HCS, and the time pattern in the nicotine condition was more similar to the placebo condition. However, no interaction involving Group was significant. SCZ again rated their subjective state more negatively overall [main effect of Group: $F_{(1,32)} = 5.84$, $p < 0.03$].

Negative affect in the ad libitum smoking condition increased from before to after cognitive testing in HCS but not in SCZ. Two factor ANOVA limited to the ad libitum smoking condition confirmed a significant Group by Time interaction [$F_{(1,32)} = 7.13$, $p < 0.02$]. This may suggest that the ability to smoke, although not the presence of nicotine (see above), prevented increases in negative affect more effectively in SCZ than in HCS.

Additional Detail on SARAT Analyses

Reaction time (RT) depended on task conditions as reported previously (11;12): RT slowed with more cued locations [main effect of Number of Cued Locations: $F_{(2,64)} = 64.5$, $p < 0.001$] and was slower for low- than high-contrast targets [main effect of Target Contrast: $F_{(1,32)} = 112$, $p < 0.001$]. The slowing with greater spatial unpredictability was more pronounced for low- than high-contrast targets [interaction of Target Contrast with Number of Cued Locations: $F_{(2,64)} = 5.12$, $p < 0.01$]. RT was overall slower in SCZ than in HCS [main effect of Group: $F_{(1,32)} = 6.52$, $p < 0.02$].

More omission errors were made with low- than high-contrast targets [main effect of Target Contrast: $F_{(1,32)} = 15.7$, $p < 0.001$]. In trials with low-contrast targets, omission errors increased with more cued locations [Target Contrast x Number of Cued Locations interaction: $F_{(2,64)} = 11.1$, $p < 0.001$]. There were no other main effects or interactions.

Additional Detail on Singleton Distractor Task Analyses

ANOVA on distractor trials only explored the effects of distractor distance from the target. Factors were Group, Drug and Distractor Distance (1, 2, 3, or 4 items from the target). A main effect of Drug was again seen on both measures, and a main effect of Group on RT. RT also displayed a main effect of Distractor Distance [$F_{(3,84)} = 17.8$, $p < 0.001$], reflecting longer RT with closer proximity to the target (Figure S4). No other effects were significant.

The Need to Concentrate as Reason for Smoking

To further test whether the 9 SCZ who endorsed that how much they smoked depended on their need to concentrate adjusted their smoking when there was a need to concentrate, we put nicotine intake in the ad libitum smoking session in relation with several indices of their usual smoking amount. This was achieved by dividing each patient's nicotine plasma concentration in the ad libitum smoking condition by (a) nicotine concentrations in the placebo session, assuming that the amount of residual nicotine in the placebo session bears relationship with nicotine intake prior to entering the experimental setting, (b) cotinine concentrations in the placebo session, assuming that the longer half life of cotinine may be a better index of nicotine intake prior to entering the experimental setting, and (c) the self-reported number of cigarettes consumed per day. Larger scores would indicate more nicotine intake relative to the usual intake when

concentration was required. There were no significant differences between the subgroups of SCZ with regard to their relative nicotine intake, but trends suggested less relative nicotine intake in the ad libitum smoking session in those SCZ who reported that their smoking amount depended on the need to concentrate [(a) $p = 0.083$; (b) $p > 0.8$; (c) $p = 0.054$], consistent with the results reported in the main body of the paper.

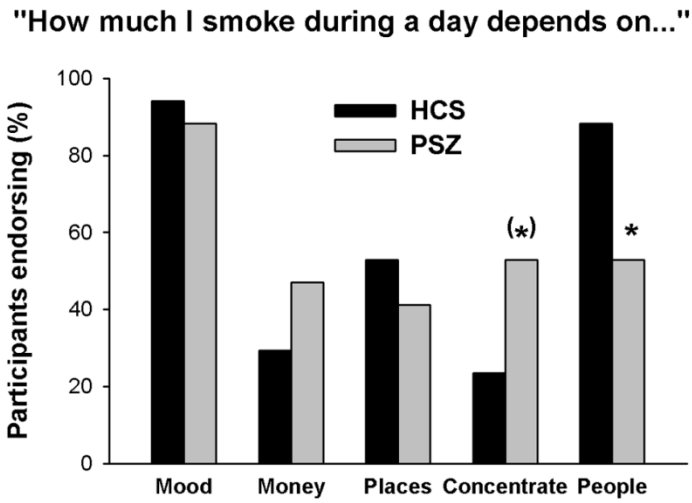

Figure S1. Percentage of participants with schizophrenia (PSZ) or healthy control subjects (HCS) endorsing that how much they smoke during a day depends on: "my mood", "how much money I have", "if I have to be in places where I can't smoke", "if I have to do things where I have to concentrate", and "if I am with other people that smoke". $*p < 0.05$, $^{(*)}p = 0.078$; χ^2 test.

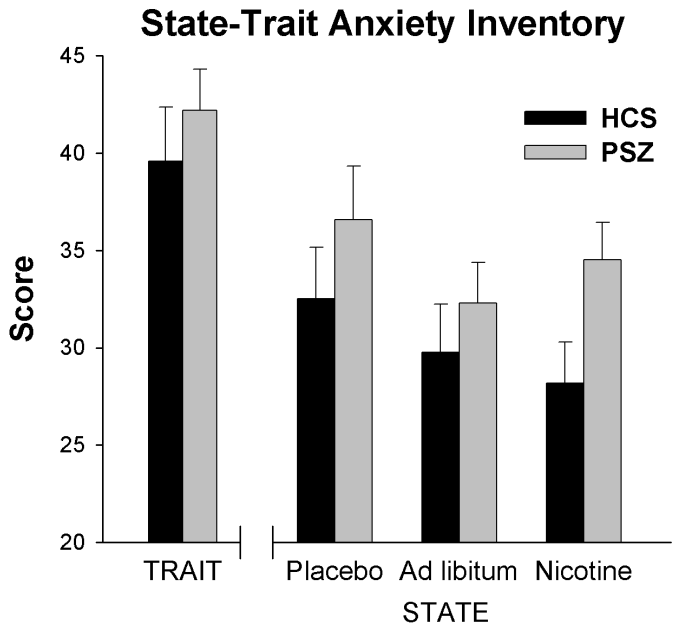

Figure S2. Average (± SEM) scores on the State-Trait Anxiety Inventory of 17 healthy control subjects (HCS) and 17 people with schizophrenia (PSZ). The trait-version was administered during the training session. The state-version was administered at the end of the placebo patch, ad libitum smoking, and nicotine patch session.

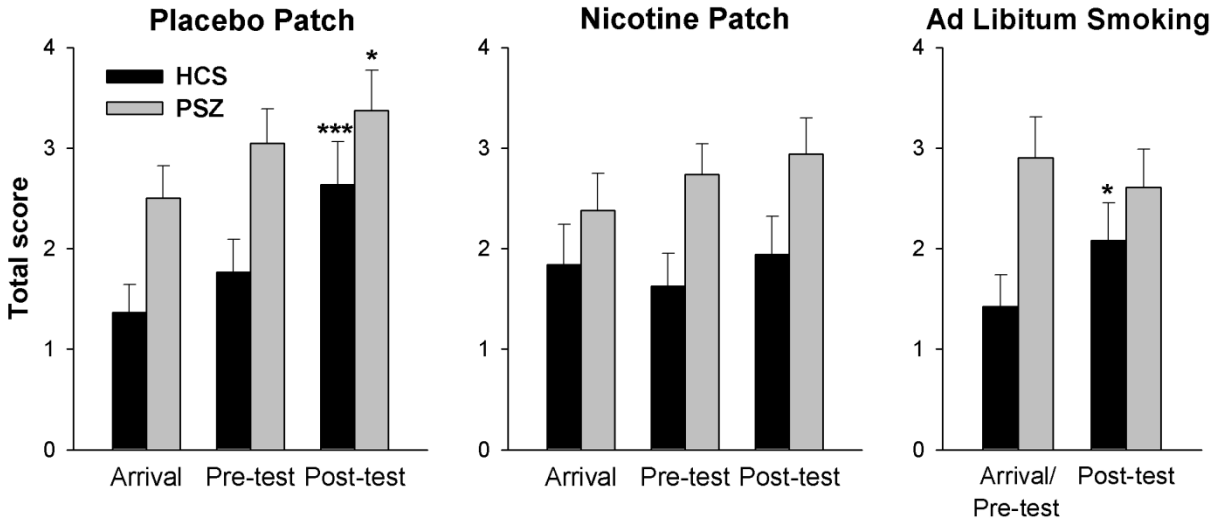

Figure S3. Average (± SEM) scores on the affect-related withdrawal scales of 17 healthy control subjects (HCS) and 17 people with schizophrenia (PSZ). The scales were administered upon arrival, after the absorption period (pre-test), and after cognitive testing (post-test). In the ad libitum smoking condition, the arrival and pre-test rating time points were identical. *$p < 0.05$, ***$p < 0001$; paired *t*-test comparison with arrival ratings.

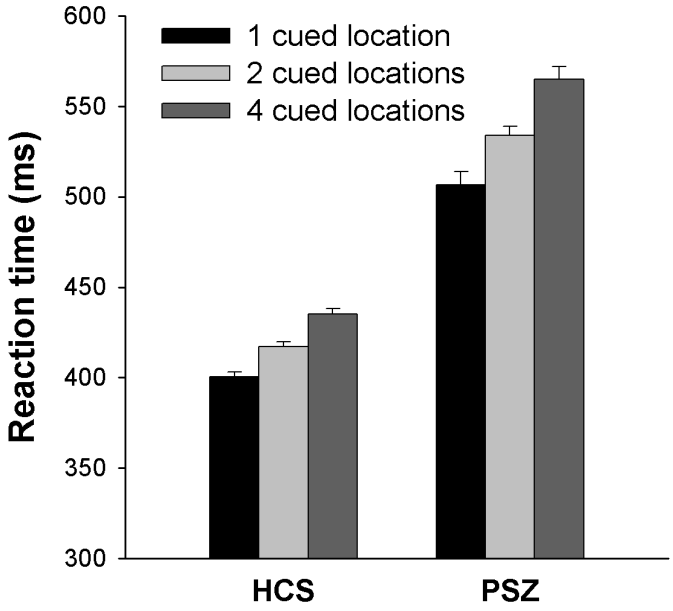

Figure S4. Average reaction time in the Spatial Attentional Resource Allocation Task as a function of group and cue condition, collapsed over drug conditions and target contrasts. The increase in reaction time with more cued locations was significantly more pronounced in people with schizophrenia (PSZ) than healthy control subjects (HCS), suggesting difficulty spreading attention broadly. Error bars reflect the SEM adjusted to remove within-group between-subject variability in average reaction time across drug conditions (13;14).

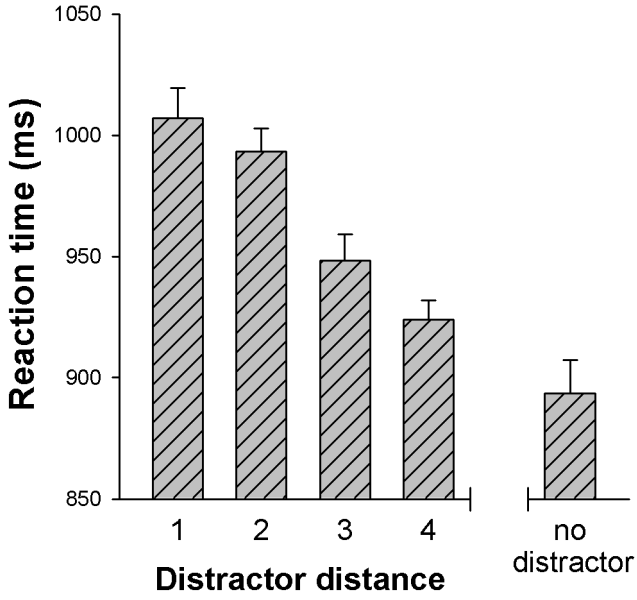

Figure S5. Reaction time in the Singleton Detection Task by distractor distance averaged over all 34 participants. Distractor distance 1 would be a color distractor located adjacent to the target stimulus. Distractor distance 4 would be the two locations most remote from the target. The two closer distractor distances produced substantially greater RT slowing relative to the no-distractor condition than the two more remote distances. Error bars reflect the SEM adjusted to remove within-group between-subject variability in average reaction time across drug conditions (13;14).

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